Journal of Molecular Biology
Volume 284, Issue 4 , 11 December 1998, Pages 1141-1151
0022-2836

doi:10.1006/jmbi.1998.2238 Cite or link using doi

Regular Article

The Fibronectin Type III Domain as a Scaffold for Novel Binding Proteins[*1, *2]

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Received 23 April 1998; revised 1 September 1998; accepted 8 September 1998. Available online 18 April 2002.

Abstract

The fibronectin type III domain (FN3) is a small autonomous folding unit which occurs in many animal proteins involving in ligand binding. The β-sandwich structure of FN3 closely resembles that of immunoglobulin domains. We have prepared a phage display library of FN3 in which residues in two surface loops were randomized. We have selected mutant FN3s which bind to a test ligand, ubiquitin, with significant affinities, while the wild-type FN3 shows no measurable affinity. A dominant clone was expressed as a soluble protein and its properties were investigated in detail. Heteronuclear NMR characterization revealed that the selected mutant protein retains the global fold of FN3. It also has a modest conformational stability despite mutations at 12 out of 94 residues. These results clearly show the potential of FN3 as a scaffold for engineering novel binding proteins.

Author Keywords: molecular recognition; combinatorial libraries; phage display; protein engineering

*1 Abbreviations used: BSA, bovine serum albumin; CDR, complementarity determining region; FN3, fibronectin type III domain; GuHCl, guanidine hydrochloride; HSQC,

WEST Search History

DATE: Monday, March 17, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=NO; OP=OR			
L40	L39 same library	3	L40
L39	L1 near10 phage	36	L39
L38	l1 with phage	44	L38
L37	L36 and loop$	18	L37
L36	L35 and l34	38	L36
L35	l1 with phage	44	L35
L34	l1 and antibod$5	5722	L34
L33	L32 and phage	455	L33
L32	l1 same antibod$5	1760	L32
L31	L30 and fibronectin$	5	L31
L30	L25 and phage	76	L30
L29	l25 and l1	6	L29
L28	L25 with l1	1	L28
L27	L25 samel1	168	L27
L26	L25 near10 l1	1	L26
L25	minibod$4 or l18	168	L25
L24	l1 and minibod$5	6	L24
L23	l1 near10 minibod$5	1	L23
L22	minobod$5 or l18	18	L22
L21	l1 near10 minobod$5	0	L21
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L19	L18 and loop$	5	L19
L18	monobody	18	L18
L17	l1 and monobody	6	L17
L16	l10 and monobody	0	L16
L15	L14 and monobody	0	L15
L14	L13 and library	22	L14
L13	L12 and phage	34	L13
L12	l10 and domain$	34	L12

L11	L10 with loop$	0	L11
L10	l1 with phage	44	L10
L9	l6 same l1	5	L9
L8	L7 and phage	3	L8
L7	L6 with l1	5	L7
L6	monobod$5	21	L6
L5	L2 and monobod$5	0	L5
L4	L2 same loop	0	L4
L3	L2 with loop	0	L3
L2	L1 near10 phage	36	L2
L1	fibronectin or fn	17283	L1

END OF SEARCH HISTORY

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FILE 'MEDLINE, CAPLUS, EMBASE, SCISEARCH' ENTERED AT 10:02:24 ON 17 MAR 2003

L1	85933	S FIBRONECTIN OR FN
L2	828	S LOOP# AND L1
L3	29	S PHAGE AND L2
L4	14	DUPLICATE REMOVE L3 (15 DUPLICATES REMOVED)
		E KOIDE SHOHEL/AU
L5	34	S E2
L6	10	S L5 AND FIBRONECTIN#
L7	8	S MONOBOD### AND FIBRONECTIN#

FILE 'STNGUIDE' ENTERED AT 10:11:00 ON 17 MAR 2003

L8	0	S MONOBOD###

FILE 'MEDLINE, CAPLUS, EMBASE' ENTERED AT 10:26:05 ON 17 MAR 2003

L9	11	S MONOBOD###
L10	9	DUPLICATE REMOVE L9 (2 DUPLICATES REMOVED)

(FILE 'HOME' ENTERED AT 09:48:11 ON 20 MAR 2003)

FILE 'MEDLINE, CAPLUS' ENTERED AT 09:48:27 ON 20 MAR 2003

L1	40249	S	FIBRONECTIN# OR FN3
L2	18	S	L1 (10A) (PHAGE DISPLAY)
L3	119	S	FN3
L4	5	S	L3 AND PHAGE
L5	10	S	L3 AND LIBRARY